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THE KARYOLOGY OF *TEREDO UTRICULUS* (GMELIN) (MOLLUSCA, PELECYPODA)

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Abstract

By counting spermatocyte and oocyte bivalents and mitotic metaphase chromosomes in cleaving eggs, we have determined both the haploid (n = 19) and the diploid numbers (2n = 38) respectively, for the species *Teredo utriculus*. An XY and XO sex-determining mechanism is absent in the species under study. Chromosomes cannot be grouped into different classes according to length. It seems that, for *Teredo utriculus*, a high number of chromosomes is not necessarily accompanied by a high amount of chromosomal DNA.

Introduction

The available karyological data on the Pelecypoda (Patterson, 1969; Hinegardner, 1974; Ahmed, 1976; Rasotto *et al.*, 1981), although still very scanty, have brought to light some interesting cytological problems: 1) in many species the male bivalents break easily and aggregate in groups (Rasotto *et al.*, 1981); thus both the number and morphology of these chromosomes are quite difficult to determine; 2) the presence of sex-chromosomes has been hypothesized for two species of the family Mytilidae: *Mytilus californianus* (Ahmed and Sparks, 1970) and *Mytilus galloprovincialis* (Rasotto *et al.*, 1981).

So far as evolution within the Pelecypoda is concerned, Patterson (1969) maintains that, as in the other groups of molluscs, the "generalized" species of this class possess lower chromosome numbers; Hinegardner (1974), on the other hand, asserts that the families Ostreidae, Pectinidae, Pinnidae, Petricolidae, and Pholadidae, considered to be more evolved on the basis of their morphological characters, have a low DNA content.

To clarify these problems we thought it useful to study the chromosomes of a member of the Teredinidae. This family includes highly specialized species and belongs to the order Eulamellibranchia cytologically not extensively analyzed. In fact, only 5 of the 59 recognized families (Grassé, 1960), have been karyologically studied (Table I).

This paper reports the analysis of male and female bivalents, and of mitotic chromosomes in cleaving eggs of the species *Teredo utriculus* (Gmelin).

Materials and Methods

For the study of spermatocyte chromosomes 30 sexually mature male specimens of *Teredo utriculus*, collected in the Gulf of Palermo, were used. The chromosome preparations were made using the well-known squashing technique (Colombera, 1970).

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TABLE I

Chromosome numbers in the order Eulamellibranchia (Mollusca, Pelecypoda)

Species name	n	Authors
Family Unionidae		
Unio sp.	16	Ahmed, 1976
Family Cardiidae		
Dinocardium robustum	12	Menzel, 1968
Cardium edule	20	Rasotto *et al.*, 1981
Cardium tuberculatum	20	Rasotto *et al.*, 1981
Family Mictridae		
Mactra sp.	18	Kostanecki, 1904
Labiosa plicatella	18	Menzel, 1968
Mulinia lateralis	18	Menzel, 1968
Family Donacidae		
Donax variabilis	18	Menzel, 1968
Family Veneridae		
Mercenaria mercenaria	19	Menzel and Menzel, 1965
Mercenaria campechiensis	19	Menzel and Menzel, 1965
Chione cancellata	19	Menzel, 1968
Saxidomus giganteus	19	Ahemd and Sparks, 1967
Saxidomus nuttalli	19	Ahemd and Sparks, 1967
Venus gallina	15	Rasotto *et al.*, 1981
Venus verrucosa	19	Rasotto *et al.*, 1981
Venerupis aurea	19	Rasotto *et al.*, 1981
Venerupis decussata	19	Rasotto *et al.*, 1981
Pitaria chione	19	Rasotto *et al.*, 1981

Unfertilized eggs of 10 females, eggs immediately after fertilization, and embryos at the 4–8 blastomere stage, obtained by fertilization *in vitro*, were treated by the method used by Colombera (1969) for the chromosome study of the species *Botryllus schlosseri* (Ascidiacea).

Observations and microphotographs of the chromosomes were performed with a Wild-phase contrast microscope.

The idiogram was constructed from photographic enlargements of the chromosomes in 7 late meiotic-II prophase plates, while the karyogram was prepared from 5 mitotic metaphase plates in embryos at 4–8 blastomere stage.

The mitotic chromosomes were interpreted according to the classification of Levan *et al.* (1964).

RESULTS

Meiotic chromosomes

From analyses of spermatocyte bivalents at diakinesis (Fig. 1a, b), the haploid number was n = 19 (Table II). The count was not difficult as broken elements were lacking.

The bivalents appeared well spaced, and intensely and homogenously stained.

In Figure 1a, the presence of chiasmata allowed different types of bivalents to be distinguished: cross-shaped with two probable sub-terminal chiasmata, one ring-shaped element with two terminal chiasmata, and rod-shaped elements in which the presence and the position of chiasmata could not be hypothesized.

TABLE II

Number of chromosomes found in the plates observed for Teredo utriculus

	n	17	18	19	20	21
Spermatocyte bivalents	frequence	2	2	42	3	1
Oocyte bivalents	frequence	2	1	35		
Late meiotic-II prophase chromosomes	frequence		2	18		
Metaphase mitotic chromosomes in cleaving eggs	2n	36	37	38	39	40
	frequence		3	25	2	

The dimensions of these chromosomes varied from a maximum of 2.7 μm to a minimum of 1.4 μm.

At late diakinesis (Fig. 1b) the cross-shaped bivalents were still present. Owing to the higher contraction of these chromosomes, the dimensions varied from 1.8 μm to 0.9 μm.

The oocyte bivalents at metaphase-I (Fig. 2) appeared well separated on the squashing plane, thus allowing an easy count (n = 19) (Table II).

In addition to the numerous cross-shaped elements with two sub-terminal chiasmata, bivalents with one terminal and one sub-terminal chiasma (Fig. 2, see arrows), and apparently achiasmatic rod-shaped elements were also visible.

The dimensions of these chromosomes varied from 3.4 μm to 1.8 μm.

In fertilized but uncleaved eggs, 20 plates, interpreted as advanced prophase at the second meiotic division, were analyzed (Fig. 3). The 19 chromosomes observed in these spreads (Table II) were rod-shaped, occasionally slightly bent, elongated, and homogenously stained. A lighter, thinner area, explained as the probable centromere position, was present in a few elements (Fig. 3, see arrows).

An average idiogram was obtained (Fig. 5) (Table III) by measuring the chromosomes of 7 plates and arranging them by length (Fig. 4, one plate is represented).

Mitotic chromosomes

Mitotic chromosomes at metaphase were observed in embryos at the 4–8 blastomere stage (Fig. 6). The chromosomes displayed different contractions in the various plates examined, and were arranged randomly on the squashing plane; from their count the diploid number resulted as 2n = 38 (Table II). In these chromosomes the kinetochore position could be identified. In fact, in some elements the sister chromatids of each chromosome were visible, while a thinner area was present in others.

An average karyotype (Fig. 8) (Table IV) was constructed by measuring and arranging the chromosomes of 5 plates (Fig. 7, three plates are represented) according to their length and to the centromere position. From its analysis it resulted that the 38 elements could be grouped into 19 pairs of autosomes, 3 of which were metacentric, 2 sub-telocentric, and 14 telocentric.

DISCUSSION

This study has determined the haploid number n = 19 and the diploid number 2n = 38 (Table II) for the species *Teredo utriculus*. The values which vary slightly from n = 19 and 2n = 38 are to be attributed to the squashing technique.

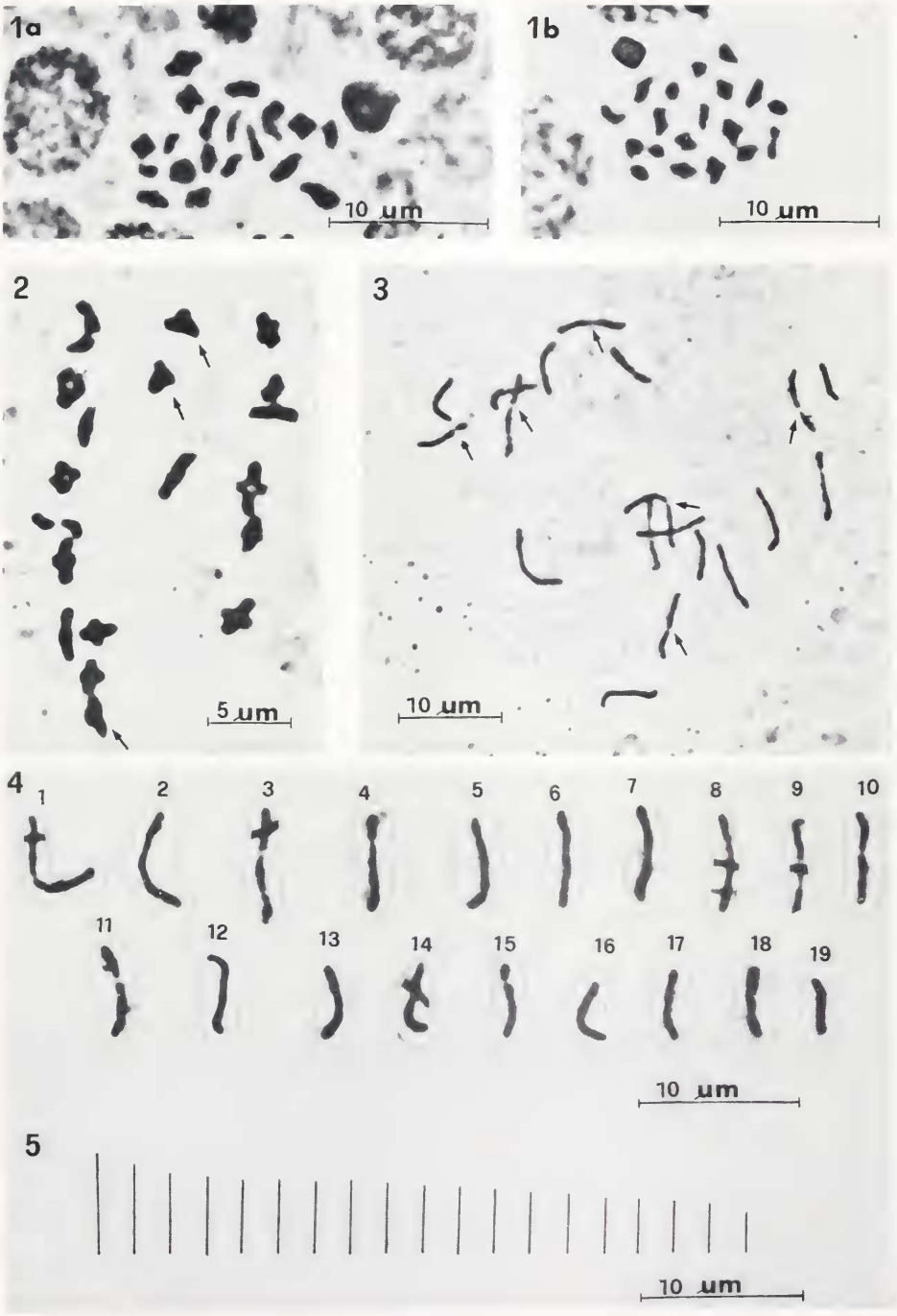

FIGURE 1a, b. Diakinetic bivalents in male gonads of *Teredo utriculus*.
FIGURE 2. Oocyte bivalents of *Teredo utriculus*.
FIGURE 3. Late prophase chromosomes at the second meiotic division of *Teredo utriculus*.
FIGURE 4. Idiogram constructed from 1 late meiotic-II prophase plate of *Teredo utriculus*.
FIGURE 5. Idiogram constructed from 7 late meiotic-II prophase plates of *Teredo utriculus*.

TABLE III

Mean length of the chromosomes and S.D. in 7 late meiotic-II prophase plates of Teredo utriculus

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Mean length in microns	6.43	5.78	5.36	4.87	4.67	4.64	4.60	4.54	4.47	4.29	4.22	4.09	3.96	3.67	3.56	3.46	3.28	3.21	2.50
S.D.	1.47	1.28	1.32	1.22	1.22	1.20	1.01	1.22	1.17	1.17	1.10	1.08	1.02	1.03	0.99	0.94	0.87	0.77	0.75

The analysis of male and female bivalents showed no heterotypic element and heteromorphism is absent in every pair of chromosomes in the karyotype. We therefore think that this species does not possess an XY or XO sex-determining mechanism.

Differentiated sex chromosomes have not been observed in any of the species of Pelecypoda cytologically examined up to now (Patterson, 1969; Ahmed, 1976; Wada, 1978; Rasotto *et al.*, 1981), apart from the species *Mytilus californianus* (Ahmed and Sparks, 1970) and *Mytilus galloprovincialis* (Rasotto *et al.*, 1981); however in both cases this assertion was based on the observation of two spermatocyte bivalents which seemed to be joined together at diakinesis.

We observed chiasmata at meiosis in both sexes, but it is very unlikely that in both spermatocyte and oocyte bivalents, all the chiasmata present were counted, due to their terminalization and the overcondensation of these chromosomes.

Furthermore, comparison of the male and female bivalents revealed the greater dimensions of the latter.

The mitotic chromosomes at metaphase appear to be arranged randomly on the squashing plane, thus excluding somatic pairing of homologous chromosomes for the species under study. However, these chromosomes appear to be peculiar for their shape, which brings to mind the "colchicinized" chromosomes (Ieyama and Inaba, 1974; Ieyama, 1975; Wada, 1978).

This characteristic, previously observed in mitotic chromosomes of spermatogonial metaphase of some Gastropods (Vitturi *et al.*, 1982), has not been confirmed in the Polyplacofora (Vitturi, 1982; Vitturi *et al.*, 1982).

The chromosomes in the idiogram and karyogram cannot be grouped into classes according to length since their dimensions vary gradually from the largest to the smallest (Tables III, IV).

If we consider the number of chromosomes, the haploid value n = 19, which characterizes the species *Teredo utriculus,* is found to be one of the highest, not only within the order Eulamellibranchia (Table I), but also within the class Pelecypoda (Rasotto *et al.*, 1981).

If, in agreement with Ahmed (1976), the basic haploid number for this class is considered to be n = 15, or a value close to that, then it seems probable that evolution within this group has proceeded not only with a decrease (Ahmed, 1976; Vitturi *et al.*, 1982) but also with an increase in the number of chromosomes (Patterson, 1969).

Finally, it is interesting to note that many species of the family Pectinidae possess 19 spermatocyte bivalents of greater dimensions (Rasotto *et al.*, 1981) than those of the male bivalents in the species analyzed here.

The finding of a low nuclear DNA content in these species (Hinegardner, 1974) leads to the supposition that there is a low DNA content in *Teredo utriculus* as well.

All this would indicate that specialization is, in this particular case, linked to a decrease in the chromosomal DNA content, thus supporting Hinegardner's hy-

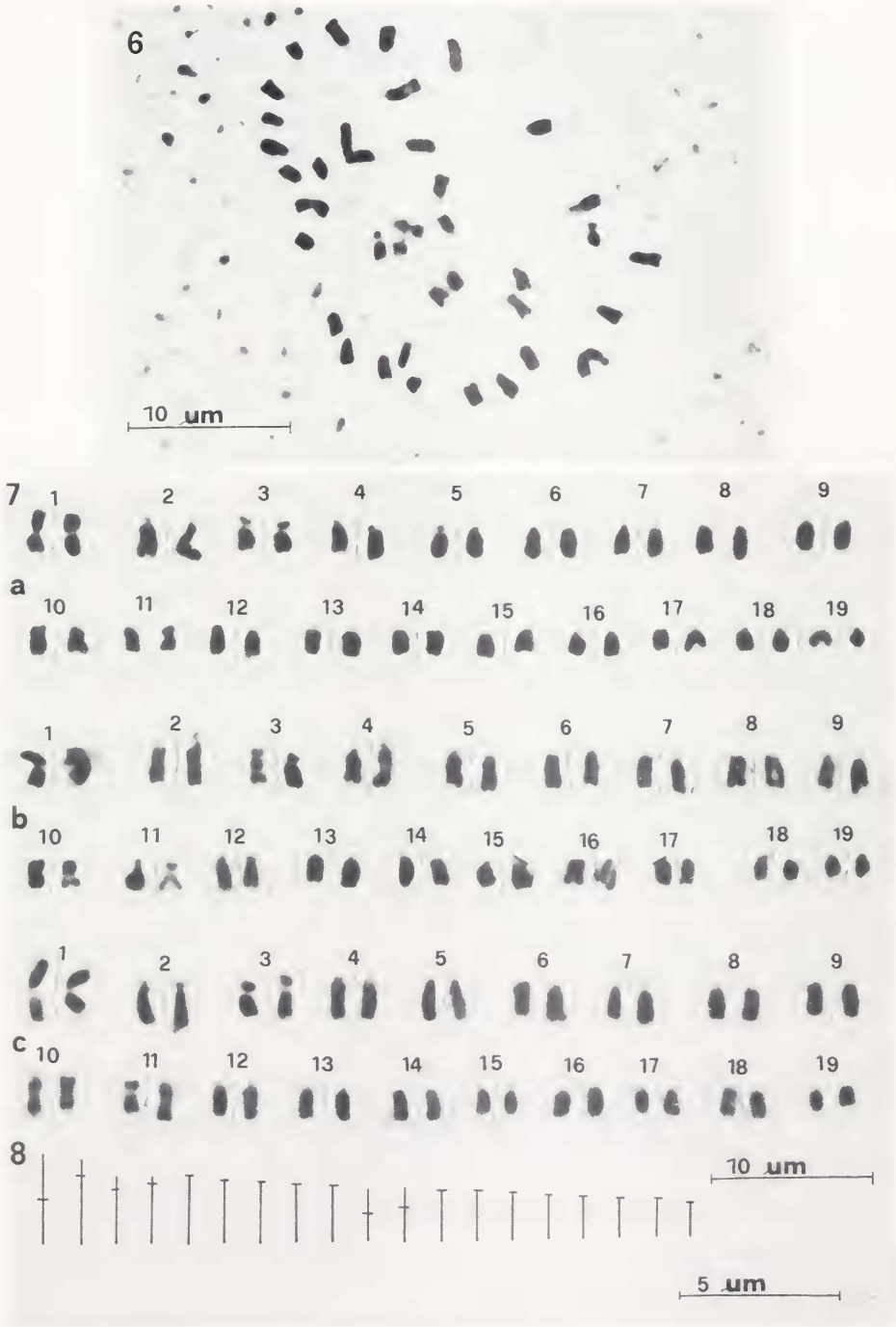

FIGURE 6. Mitotic metaphase plate in cleaving eggs of *Teredo utriculus*.

FIGURE 7. Three arrangements of mitotic metaphase chromosomes in cleaving eggs of *Teredo utriculus*.

FIGURE 8. Karyogram constructed from 5 mitotic metaphase plates in cleaving eggs of *Teredo utriculus*.

TABLE IV

Mean length and arm ratio of the chromosomes of 5 mitotic metaphase plates in cleaving eggs of Teredo utriculus

Chromosome pairs	Mean length in microns	S.D.	Arm ratio mean	Centromere position
1	2.86	0.53	1	M
2	2.59	0.33	5.3	ST
3	2.13	0.22	3.7	ST
4	2.09	0.28	8.2	T
5	2.09	0.28	∞	T
6	1.93	0.21	∞	T
7	1.86	0.15	∞	T
8	1.82	0.12	∞	T
9	1.82	0.12	∞	T
10	1.77	0.15	1	M
11	1.72	0.19	1.7	M
12	1.68	0.22	∞	T
13	1.66	0.18	∞	T
14	1.57	0.22	∞	T
15	1.43	0.15	∞	T
16	1.37	0.13	∞	T
17	1.29	0.15	∞	T
18	1.22	0.18	∞	T
19	1.11	0.25	∞	T

pothesis that such a mechanism is present in all classes belonging to the phylum Mollusca.

At any rate, as has already been suggested for the family Petricolidae (Pelecypoda) (Rasotto *et al.*, 1981), for the Polyplacofora (Vitturi, 1982) and for the Mesogastropoda (Mollusca, Prosobranchia) (Vitturi and Catalano, in press) it appears that, for *Teredo utriculus* as well, a high number of chromosomes is not necessarily accompanied by a high amount of chromosomal DNA.

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